10/537149

Applicants

Ying Wu

JC17 Rec'd PCT/PTO 02 JUN 2005

Marinus Gerardus Johannes Van Beuningen

Alan Chan

Appl. No.

Not Yet Assigned (U.S. National Phase of PCT/EP2003/013601)

Filed

Herewith

Amendments to the Claims:

Please cancel Claims 1-35 without disclaimer or prejudice to applicants' right to pursue the subject matter of these claims in future divisional or continuation application, and add new Claims 36-62 as set forth below.

1-35 (cancelled).

- 36. (New) A method for hybridization of probes onto immobilized genomic DNA comprising the steps of:
 - (a) providing intact genomic DNA and denaturing said intact genomic DNA;
- (b) immobilizing said denatured intact genomic DNA onto a matrix; said matrix comprising pore sizes within a range of 0.6 μ m to 2 μ m including the outer limits;
- (c) providing a set of probes and passing said probes through said matrix under conditions favoring hybridization of the probes to its complementary sequence in said intact genomic DNA; and
- (d) washing off non-hybridized probes through said matrix, leaving formed hybridized intact genomic DNA/probe complexes for further analysis.
- 37. (New) The method according to claim 36, wherein said denatured intact genomic DNA is permeated within said matrix.
- 38. (New) The method according to claim 36, wherein said probes are passed through said matrix by at least one cycle of alternating downwards and upwards flow.

Ying Wu

Marinus Gerardus Johannes Van Beuningen

Alan Chan

Appl. No.

Not Yet Assigned (U.S. National Phase of PCT/EP2003/013601)

Filed :

- 39. (New) The method according to claim 36, wherein said washing step is carried out by passing through said matrix a wash fluid by at least one cycle of downwards flow.
- 40. (New) The method according to claim 36, wherein said matrix is a membrane.
- 41. (New) The method according to claim 40, wherein said membrane comprises a 3D network structure.
- 42. (New) The method according to claim 41, wherein said network structure is a flow-through structure.
- 43. (New) The method according to claim 41, wherein said network structure is a fibre network structure.
- 44. (New) The method according to claim 43, wherein said fibre is of vegetable origin.
- 45. (New) The method according to claim 44, wherein said fibre is cellulose.
- 46. (New) The method according to claim 36, wherein the matrix allows for a flow rate comprised between 50mm/30min and 250mm/30min including the outer limits.

Ying Wu

Marinus Gerardus Johannes Van Beuningen

Alan Chan

Appl. No.

Not Yet Assigned (U.S. National Phase of PCT/EP2003/013601)

Filed

- 47. (New) The method according to claim 36, wherein said matrix is activated with an affinity conjugate.
- 48. (New) The method according to claim 47, wherein said affinity conjugate is chosen from the group comprising poly-L-lysine, poly-D-lysine, 3-aminopropyl-triethoxysilane, poly-arginine, polyethyleneimine, polyvinylamine, polyallylamine, tetraethylenepentamine, ethylenediamine, diethylenetriamine, triethylenetetramine, pentaethylenehexamine and hexamethylenediamine.
- 49. (New) The method according to claim 48, wherein said affinity conjugate is poly-L-lysine.
- 50. (New) The method according to claim 36, wherein said probes are flanked by primer binding sequences.
- 51. (New) A method for target nucleic acid detection and quantification in an intact genomic DNA sample comprising the steps of:
 - (a) providing intact genomic DNA and denaturing said intact genomic DNA;
 - (b) performing a hybridization according to the method of Claim 36;
- (c) recovering hybridized probes; and essentially simultaneously amplifying any recovered probe using a single primer pair, each member of said primer pair binding to each recovered probe onto the respective flanking primer binding sequences of said probe; and
- (d) qualitatively and quantitatively analyzing the recovered amplified probes of step (c).

Ying Wu

Marinus Gerardus Johannes Van Beuningen

Alan Chan

Appl. No.

Not Yet Assigned (U.S. National Phase of PCT/EP2003/013601)

Filed

- 52. (New) The method according to claim 51, wherein the analysis of step (d) is by microarray analysis.
- 53. (New) The method according to claim 51, wherein each probe is flanked 5' and 3' by primer binding regions with said 5' and 3' flanking primer binding sequences being the same or substantially the same for each probe.
- 54. (New) The method according to claim 51, wherein said amplification of step (c) is a quantitative amplification.
- 55. (New) The method according to claim 54, wherein said amplification is by means of polymerase chain reaction.
- 56. (New) The method according to claim 51, wherein the amplified probes are provided with a label.
- 57. (New) The method according to claim 56, wherein said label is a fluorescent label.
- 58. (New) A device for flow-through hybridization of probes onto immobilized intact genomic DNA comprising a well holder, said well holder comprising one or more round wells with a fixed diameter, said wells exposing a fibre network matrix, said matrix comprising pore sizes within a range of $0.6 \mu m$ to $2 \mu m$ including the outer limits; wherein said matrix permits immobilization of intact genomic DNA and which allows hybridization of said immobilized intact genomic material with probes by flow-through hybridization.

Ying Wu

Marinus Gerardus Johannes Van Beuningen

Alan Chan

Appl. No.

Not Yet Assigned (U.S. National Phase of PCT/EP2003/013601)

Filed

- 59. (New) The device according to claim 58, wherein said matrix permits permeation of intact genomic DNA.
- 60. (New) An apparatus for flow-through hybridization of probes onto immobilized genomic DNA comprising:
 - (a) a device according to claim 58;
- (b) means for addition of a controlled amount of fluid to at least one of the wells of the device as described in (a);
- (c) means for applying and/or maintaining a controlled pressure difference over the matrix in each of the wells.
- 61. (New) A kit for flow-through hybridization of probes onto immobilized intact genomic DNA comprising:
 - (a) a device according to claim 58; and
 - (b) instructions.
 - 62. (New) A kit according to claim 61, additionally comprising:
- (a) a set of probes, wherein each probe is flanked 5' end 3' by primer binding regions with said 5' and 3' flanking primer binding sequences being the same or substantially the same for each probe;
- (b) a single primer pair, each member of said pair being complementary to a primer binding region;
- (c) optionally amplification components allowing the amplification of any recovered hybridized probe; and
- (d) optionally a microarray, said microarray allowing analysis of the hybridization results.